attached antigen is important for B cell signaling. B cells gather antigen from a variety of sources which may have different physical characteristics such as mobility, stiffness or topography. However, the effect of these parameters on BCR clustering and signaling activation is not understood. We have studied the interaction of B cells with BCR ligand coated surfaces to investigate the physical parameters affecting BCR microcluster formation, cell spreading and signaling activation. Using high-resolution TIRF microscopy of live cells, we followed the movement and spatial organization of BCR clusters and the dynamics of actin as well as the associated signaling on surfaces with different physical properties. Using glass and lipid bilayer surfaces, we found that both immobile and mobile ligands are able to crosslink BCRs and induce clustering. However, B cells interacting with mobile ligands (on lipid bilayer) displayed greater signaling than those interacting with immobile ligands (on glass). Quantitative analysis revealed that mobile ligands enabled BCR clusters to move farther and merge more efficiently than immobile ligands. We also investigated the effect of substrate topography and substrate stiffness on BCR and actin dynamics. Quantitative analysis showed that these parameters were associated with differences in actin remodeling and spreading behavior. Our results indicate that B cells are highly sensitive to a range of physical parameters during cell spreading and signaling activation.

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The Actin Crosslinking Protein Palladin Modulates Force Generation and Mechanical Sensing of Tumor Associated Fibroblasts

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Cells organize actin filaments into higher-order structures by regulating the composition, distribution and concentration of actin crosslinkers. Palladin is an actin-crosslinking protein that is found in the lamellar actin network and stress fibers, two actin structures critical for mechanosensing of the physical environment. Palladin also serves as a molecular scaffold for alpha-actinin, a key actin crosslinker. By virtue of its close interactions with actomyosin structures in the cell, palladin may play an important role in cell mechanics. However, the role of palladin in cellular force generation and mechanosensing has not been studied. In this study we use human pancreatic tumor associated fibroblasts (TAFs) to investigate the role of palladin in regulating the plasticity of the actin cytoskeleton and cellular force generation in response to alterations in substrate stiffness. Traction force microscopy revealed that TAFs are sensitive to substrate stiffness as they generate larger forces on substrates of increased stiffness. Contrary to expectations, knocking down palladin increased the forces generated by cells, and also inhibited the ability to sense substrate stiffness for very stiff gels. This was accompanied by significant differences in the actin organization and adhesion dynamics of palladin knock down cells. Perturbation experiments also suggest altered myosin activity in palladin KD cells. Our results suggest that the actin crosslinkers such as palladin and myosin motors coordinate for optimal cell function and to prevent aberrant behavior as in cancer metastasis.

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Mechanical Stress in Actinin and Actin in Stem Cells

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cpstFRET is a FRET based force sensor designed to be modulated primarily by the angle between the donor and acceptor. It is physically smaller, less invasive, and has greater dynamic range and better signal-to-noise behavior than the linear probes. Using this sensor with FRET polarization imaging we measured the gradients of constitutional stresses in time and space for actinin and actin in HEK and MDCK cells. We created eight stable cell lines with these probes. Then we derived stem cells from the cell lines and measured stress changes during differentiation and dedifferentiation. We calculated FRET with the parallel/perpendicular polarization images of FRET. Because the FRET signal is always more depolarized, high ratios represent low FRET and high stress, while low ratios represent high FRET and low stress. We induced HEK and MDCK cells into embryoid bodies (EB) which is the characteristic morphology of induced pluripotent stem cells and cancer stem cells. We verified the stem cell in the EBs with alkaline phosphatase (AP) staining. Stem cells in EBs derived from HEKs showed higher AP activities than those derived from MDCK cells. All stem cells showed escalated stress in both actinin and actin relative to the parent. Stress was higher in stem cells of HEK origination. After we removed the EB-induction factors, these stresses declined as the stem cells differentiated into HEK or epithelial cells. We also induced the stem cells differentiate into neurons. The cell body showed low stress while axon extrusions showed increased stress in actin and actinin. The significant stress changes in stem cells and differentiated descendants hint at the potential of inducing pluripotent stem cells through changing cell mechanics. The data also shows that stem cell differentiation involves changes in internal stresses as well as changes in biochemistry.

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Single Molecule Mechano-Memory

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Understanding the spatial distribution of individual adhesion bonds and the tension exerted on them is crucial for understanding whole cell adhesion behavior. Here, we introduce a new class of molecular force sensors to record cellular adhesion events at the single-molecule level. A DNA structure was designed that responds to mechanical perturbation above certain threshold tension and maintains a memory of that perturbation. We name this feature "single-molecule mechano-memory" (smMM). The smMM sensor undergoes conformational changes under tension and is kinetically trapped under a new conformation. Single-molecule force spectroscopy and fluorescence spectroscopy were performed to characterize the activation force as well as memory life time. We show that in the absence of mechanical perturbation the smMM sensor is well folded and stable. In the presence of tension above ~35 pN, the sensor is converted to the unfolded "memory" state in which it remains kinetically trapped for an average of 25 seconds. Both activation force and life time of the sensor can be tuned by its DNA sequence. As a proof of concept for this class of sensors, smMM sensors were coated on a surface where cell adhesion takes place. Individual adhesion events are detected using fluorescently-labeled oligonucleotide probes to mark unfolded sensors.

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DNA-Based "Digital" Tension Probes with Pn Sensitivity Reveal Early Cell Adhesion Mechanics at the Single Molecule Level

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Mechanical forces are involved in important processes such as cell division, migration and gene expression, signifying their critical importance to living systems. However, the lack of methods to visualize cellular forces at the molecular scale has hampered the study of mechanotransduction. To address this need, we developed a new class of DNA-based molecular tension fluorescence microscopy (MTFM) probes that function as a reversible digital switch, and are ideally suited to investigate the pN-range forces applied by individual integrins. We show that focal adhesion maturation involves an increase in tension per molecule coupled with recruitment of a greater density of integrins. By engaging cells to sensor chips presenting mixtures of spectrally-encoded probes with different mechanical responses, we find that integrins display both chemical and mechanical specificity at the single-molecule level. Integrins show mechanical preference for more rigid ligands within nascent adhesions, thus suggesting focal adhesions function as rigidity sensors at the single integrin level. Moreover, this observation may be related to the "catch" bond model, where the integrin-ligand bond lifetime increases under a mechanical load.

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Buckling of a Physically-Constrained Growing Epithelium

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In growing tissue, cell proliferation and tensile/compressive forces generated by growth are essential for controlling shape and size of organs during development (Mammoto T and Ingber DE 2010). One example of a system where these parameters may have a critical influence is an early stage of embryogenesis, namely gastrulation. During gastrulation, the spherically shaped epithelium invaginates and forms a tube in the lumena of the embryo that later will be the digestive tube. The invagination of the epithelia cells is promoted by genetic factors. However, recent theoretical models proposed that compression of the embryo in a spherical shell could promote gastrulation by inward buckling (Hannezo E et al. 2011, Tamulonis C et al. 2011). As embryo is not easily amenable to forces and shape measurements, to study the coupling between mechanical forces/confinement and epithelium shape and proliferation, we create an epithelial spherical monolayer in the elastic shell using a technique of cell encapsulation in the shell made out of alginate. As the elastic modulus of alginate is known, small deformation of the shell will allow us to measure compression within the epithelium (Alessandri K et al. 2013). We then follow the growth of the epithelium through time and observe its buckling. We address the questions of how the pressure builds up in the growing epithelia enclosed in